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14. ABSTRACT

Purpose and scope: A large genetic association study is being conducted, to examine relationships of prostate cancer risk with polymorphic variation in a series of selected candidate genes that are involved in pathways determining the synthesis of IGF-I and IGF-binding proteins, as well as biological response to IGF-I. The study is being performed within a large Swedish case-control study ("CAPS").

Progress report: We completed, as planned, the completion of DNA distribution at IARC. We have selected haplotype tagging SNPs to be analyzed for all candidate genes, and have completed about half of all genotyping assays for the prostate cancer cases and control subjects. We have also completed a linked database, containing data on tumour grade, stage and serum PSA levels, for all prostate cancer cases. Plasma assays of IGF-I and IGFBP-3 have been delayed, due to logistic complications, and will be performed in year 3.

Conclusions: For the genetic component of our project we are entirely on schedule. We have commenced the genotyping of the genetic variation in the candidate genes of the IGF1 pathway and anticipate the completion of the genotyping component of this study, as well as IGF-I and IGFBP-3 assays, by the end of 2005. Statistical analysis of relationships between genetic variants, plasma IGF-I and IGFBP3 levels, and prostate cancer risk, will be completed in 2006.

15. SUBJECT TERMS
Insulin-like growth factor (IGF-I), Genetic Polymorphisms, Epidemiology, Prostate Cancer Risk

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Introduction

Evidence is rapidly accumulating that insulin-like growth factor-I (IGF-I) can enhance the development of tumors in different organs. Studies *in vitro* have shown that IGF-I inhibits apoptosis and stimulates cell proliferation in a wide variety of cell types. Furthermore, tumor development can be strongly enhanced in animals or organs that have been genetically or otherwise manipulated to either over express IGF-I or the IGF-I receptor, whereas animals made deficient in IGF-I are protected. Experiments with IGF-I$^{-/-}$ null mice have shown that normal IGF-I levels are required for prostate gland development, and transgenic mice expressing human *IGF1* in basal epithelial cells of the prostate have a high spontaneous incidence of prostatic tumors. In men, several prospective cohort studies and case-control studies have shown an increased prostate cancer risk among men who have elevated plasma IGF-I levels – expressed either as absolute concentrations, or relative to levels of IGFBP-3, IGF's major plasmatic binding protein.

Most of IGF-I and IGF-binding proteins in the circulation originates from the liver, but all peptides are also formed in other organs, including the prostate, where they exert paracrine and autocrine effects. Circulating IGF-I, as an endocrine factor, can diffuse towards its target tissues. In addition, IGF-I synthesis by the liver and many other organs is very much controlled by the same endocrine and nutritional factors. The major endocrine stimulus to IGF-I synthesis, in liver and many other tissues, is provided by growth hormone (GH). Thus, elevated IGF-I in blood most likely reflects an elevated pituitary GH secretion, and most likely indicates also elevated levels in other tissues where GH also provides the principal stimulus to IGF-I synthesis.

Given the increasing evidence that elevated IGF-I may enhance cancer development, it is important to understand what factors can lead to elevated IGF-I in the circulation and tissues. Besides nutritional status (Kaaks & Lukanova, 2001), heritability studies have shown that, at least in western, well-nourished populations, a large part (40-60 %) of variation in IGF-I is (co-) determined by genetic factors (Hong et al., 1996; Harrela et al., 1996; Verhaeghe et al., 1996). So far, however, no studies have been published, reporting a comprehensive search for polymorphisms in a full panel of genes involved in regulating IGF-I synthesis, and correlating such a panel with inter-subject variations in IGF-I and IGFBP-3 levels.

Besides the genes for IGF-I (*IGF1*) and IGFBP-3 (*IGFBP3*), major candidate genes to be examined are those involved in the pituitary release or biological action of growth hormone — the primary physiological stimulus for the synthesis of both IGF-I and IGFBP-3. This latter includes the genes for somatostatin (*SST*) and its receptors (*SSTR1-5*), pituitary-specific transcription factor (or POU-domain class 1 transcription factor 1 (*POU1F1*); growth hormone (*GH1*) and its receptor (*GHR*), growth hormone releasing hormone (*GHRH*), and the GHRH receptor (*GHRHR*). Ghrelin (*GHRL*), a recently identified new peptide hormone produced by endocrine cells in the stomach, also stimulates growth hormone secretion. It is the first identified natural ligand for a previously cloned growth hormone secretagogue receptor (*GHSR*) which is present in the pituitary gland and the hypothalamic region of the brain. In the circulation, IGF-I and a large percentage of IGFBP-3 are bound to a third peptide, referred to as Acid Labile Subunit (*IGFALS*) which has a key role in stabilizing the circulating pool of these peptides, and in regulating IGF-I release towards tissues. For each of these genes, polymorphisms that change gene expression or protein function can be expected to result in a relative increase or decrease in circulating IGF-I or IGFBP-3 levels.

The specific aims of our project are the following:

- to examine associations of prostate cancer risk with polymorphic variants (single nucleotide polymorphisms [SNPs] or their haplotypes) of selected candidate genes that may determine the synthesis and circulating levels of IGF-I, or and biological response to IGF-I;
- to confirm that elevated IGF-I levels, as absolute concentrations or expressed relative to concentrations of IFBP-3, are associated with an increased risk of prostate cancer; and
- examine whether associations of prostate cancer risk with polymorphic gene variants can be explained, at least in part, by associations of the same gene variants with circulating IGF-I or IGFBP-3 levels.

Table 1. Candidate genes for studies of association with plasma IGF-I, IGFBP-3, and prostate cancer risk.

Gene	Name and Function of gene product
IGF-I	Insulin-like growth factor-I
GH1	Growth hormone: Main stimulus for synthesis of IGF-I and IGFBP-3
GHR	Growth hormone receptor: mediates GH effects
GHRH	Growth hormone releasing hormone: stimulates pituitary GH release
GHRHR	Growth hormone releasing hormone receptor; Mediates GHRH effects
SST	Somatostatin; inhibits pituitary GH release
SSTR1 – SSTR5	Somatostatin receptors, types 1 - 5; mediate SST effects on pituitary GH release
POU1F1	pituitary-specific transcription factor; crucial for pituitary GH synthesis
IGF1R	IGF-I receptor
GHRL	Ghrelin
GHSR	Growth hormone secretagogue receptor
IGFALS	IGF binding protein, acid labile subunit
IGFBP1 - 6	IGF-binding proteins 1 to 6

Progress report, year 2.

For year 2, our tasks, as in the "Statement of Work" of our original grant application, were the following:

Task 5 (months 10-12): Measurement of assays of IGF-I and IGFBP-3 in plasma of prostate cancer cases (n=1000) and controls (1200). These assays have not yet been performed, because of delays in logistics of retrieving plasma samples from the central CAPS biobank in Umeå (Sweden) and shipment of these samples to the hormone assay laboratory at IARC (Lyon, France). An additional reason was that the hormone assay laboratory at IARC was heavily booked for assays in other, parallel projects. Shipment of the plasma samples and biochemical assays are now scheduled for year 3, and before the end of 2005.

Task 6 (Months 15-28): Genotyping of cases and controls for polymorphisms in genes related to the IGF system. An extensive search was made in the now publicly accessible "HapMap" database, which provides very detailed information about the presence of genetic variants and their linkage disequilibrium patterns, in genes throughout the genome. This search, combined with our own previous work for the identification of SNPs, has allowed us to make a more exhaustive screen of the genetic and haplotypic that exists in the candidate genes of the IGF1 pathway than initially envisaged. By following the 'haplotype tagging' SNP approach, discussed in the year one progress report, allows greater efficiency in our genotyping strategy. One clear

example is the GHR gene in which, following the protocol outlined by Stram et al. (2003), a total of 113 SNPs can be tested by only 19 htSNPs, with only minimal loss of information (due to the fact that SNPs are often in linkage disequilibrium, making measurement one SNP a measurement of others by proxy) (Stram et al., 2003) (**Table 2**).

Table 2. htSNPs' selected and genotyping completed in the CAPS study.

Genes	Genome size (kb)[Ψ]	SNP's in gene region[t]	htSNPs selected*	SNPs genotyped
IGF1	128	44	11	8
IGFBP1	24	10	6	6
IGFBP3	70	18	6	6
IGFALS	9	6	3	2
GHR	447	113	19	6
SST	46	18	4	
SSTR1	18	6	4	
SSTR2	16	8	4	
SSTR3	40	29	7	
SSTR4	8	3	3	
SSTR5	19	9	7	
GHRH	10	2	2	
GHRHR	37	13	8	
IGFBP2	66	12	8	
IGFBP5	29	9	6	
IGFBP4	18	7	7	
IGFBP6	26	6	5	

[Ψ]Genomic size including blocks of LD (defined by Gabriel et al., 2002 method) that may partially overlap with genomic sequence

[t]Number of confirmed polymorphic SNPs contained in the gene region (and in LD blocks that cover the gene) identified from the HapMap initiative and IARC SNP discovery work

*Selected on the basis of an R2h>0.8 for SNPs inside haplotype blocks (defined by Gabriel et al., 2002 method) and R2s>0.8 for SNPs falling in-between or just outside haplotype blocks if that distance is less than 10kB (Stram et al., 2003)

At the end of May 2005, we have completed approximately half of all htSNPs, focusing first on the IGF1, IGFBP1 and IGFBP3, IGFALS and GHR genes. Concordance with Hardy Weinberg equilibrium and QC analysis have been completed for these first genes, and preliminary analysis of this data for disease outcomes has begun. For the remaining genes (IGFBP2, IGFBP4, IGFBP5, IGFBP6, SST, SSTR1-5, GHRH, PouF1, GHRHR, GHRL, GHSR), htSNPs have been selected and assay development, optimization and genotyping will be performed in the remaining part of 2005.

Task 7 (Months 13-15): Linkage of study set to primary registry of four regions to obtain data on tumor grade, stage and serum PSA levels as well as date and type of cancer treatment.

Tasks 8 and 9 (Months 15-36): Statistical analysis of associations between genetic polymorphisms, plasma levels of IGF-I, IGFBP-3 and risk of prostate cancer. We have started performing preliminary statistical analyses on the relationship of prosate cancer risk with

polymorphic variants in the IGF1, IGFBP1, IGFBP3, IGFALS and GHR genes. By contrast, we have not yet started any analyses on relationships of polymorphisms or prosate cancer risk with plasma IGF-I and IGFBP-3 levels, as these assays have not been performed yet.

Task 10 (Months 17-36): Interpretation of data, writing of reports. A first start has been made with the drafting of two first articles: one on the relationships of prostate cancer risk with polymorphic variants in the IGF1, IGFBP1, IGFBP3, and IGFALS genes, and one on the relationships of prostate cancer risk with polymorphic variants in the GHR gene.

Key research accomplishments, year 2

Accomplishments of year 2 include:

- Correlation of the SNPs discovered at IARC and those genotyped by the HapMap initiative
- Selection of haplotype tagging SNPs (htSNPs) for genes to be genotyped. Optimization of TAQMAN assays for the htSNPs.
- Commencement of genotyping in the case/control series at IARC in 4865 individuals for the genes IGF1, IGFBP1, IGFBP3, IGFALS and GHR, and completion of assays for the first 40 percent of htSNPs to be typed (in May 2005, approximately 136,000 genotypes have been completed).
- Establishment of a linked database containing data on tumour grade, stage and serum PSA levels has been assembled and distributed among the collaborating partners.

Reportable outcomes

The study has not yet resulted in reportable outcomes (this will be the case in year 3)

Conclusions

We have put into place the samples and their need to complete the CAPS studies investigation of the genes of the IGF1 pathway. The genes have been sufficiently "tagged" with htSNP's and for the IGF1, IGFBP1, IGFBP3, IGFALS and GHR genes these assays have been designed, optimized and nearly completed. For the other genes, analyses for SNP tagging have also been nearly completed, and assays are planned to be genotyped before the end of 2005. Assays of plasma IGF-I and IGFBP-3 concentrations have been delayed, but are scheduled before the end of 2005.

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Appendices

None